Week 14: 7. September 2015 – 11. September 2015

7. September 2015

1) Preculture for purification of MEDH2
    - Inoculate a 100 ml LB+Kan preculture and incubate at 37 °C shaking at 220 rpm overnight.

8. September 2015

1) Expression of MEDH2 in *E. coli*

- Inoculate 6 mainculture of 1 liter LB+Kan with 10 ml of the preculture
- Incubate until OD600 reaches 0.8.
- Take a 1 ml sample in an uninduced stage.
- Add 1 ml 0.25 M IPTG to each 1L and incubate at 18 °C shaking at 220 rpm overnight.

9. September 2015

1) Cell lysis to obtain expressed MEDH2

- Harvest the expression cultures by centrifugation at 5500 rpm at roomtemperature for 20 minutes .
- Resuspend the cell pellet in 15 ml imidazole buffer
- Sonicate 3 x 30 sec at an amplitude of 60. Incubate 1 minute on ice between each sonication step.
- Centrifuge the lysate at 13.000 rpm at 4 °C for 30 minutes
- Keep the supernatant.

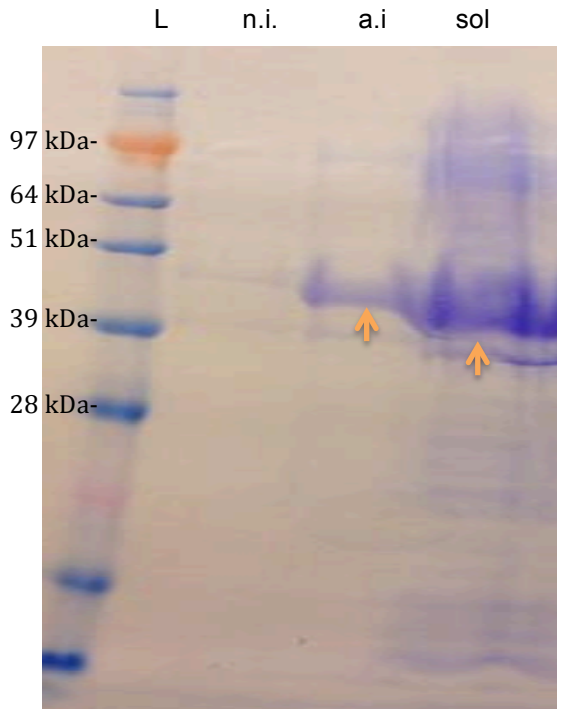

Figure 1: Expression analysis of MEDH2. Coomassie staining of MEDH2 used for Ni-NTA Purification. Samples were taken in an uninduced stage of protein expression (n.i.) and after expression at 18 °C overnight (a.i.). Cells were lysed by sonication and the pooled soluble fraction of the cultures was analyzed (sol). Theoretical molecular weight: MEDH2- 40.15 kDA. Orange arrows indicate expressed protein.

2) Ni-NTA affinity chromatography of heterologous expressed MEDH2 in E. coli

- Equilibrate the Ni-NTA with 10 mM imidazolebuffer.
- Mix the equilibrated Ni-NTA with the obtained supernatant from step 1) and gently roll for 40 minutes.
- Transfer the mixture to an Econo-column (BIORAD) and collect the flowthrough.
- Wash with 100 ml 10 mM imidazolebuffer and collect the fraction.
- Wash with 30 ml 50 mM imidazolebuffer and collect the fraction.
- Elute with 15 ml 300 mM imidazolebuffer and collect the eluate.
- Analysis of the obtained fractions on SDS-Page

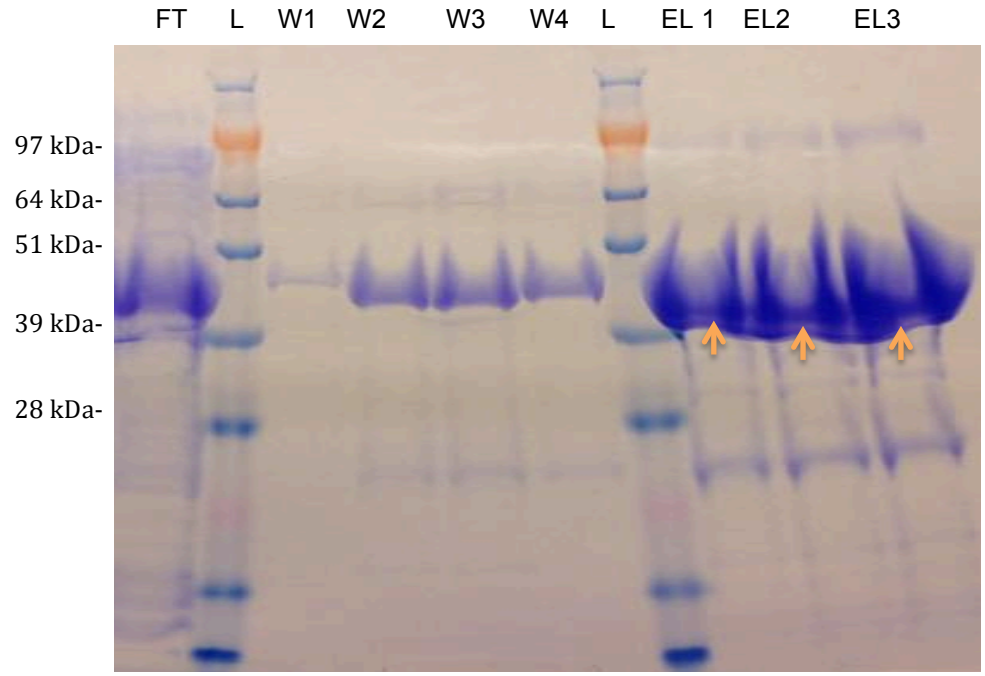

Figure 2: Ni-NTA purifcation of MEDH2. Samples were analyzed by SDS-Page. Samples oft he Flowtrough (FT), of the washingsteps (W1-4) and of the eluates (E1-3) were analyzed. Theoretical molecular weight: MEDH2-40.15 kDa. Orange arrows indicate the protein.

2) Dialysis of eluate fraction

- Combine the eluate fractions xy and dialyse against dialysis buffer for 2 hours, changing the buffer after 1 hour.

3) Protein concentration determination by Bradford

- Protein concentration was determined by Bradford assay
- Final protein concentration: 13 mg/ml

10. September 2015

1) NAD+ dependent methanol dehydrogenase *in vitro* assay

- Perform the reaction in a total volumne of 1 ml
    - 26 ug purified MEDH2
    - 10 mM Methanol
    - 5 µM NAD+
    - Fill up to 1 ml with 50 mM K_2HPO_4 buffer (pH 7.4) + 5 mM $MgSO_4$
- Blank against the reaction mix without added MEDH2
- Measure the absorption at 340 nm over 20 minutes
- No results were detectable